

# Kongeriget Danmark

Patent application No.: PA 2002 01898

Date of filing: 11 December 2002

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Title: Detergent composition

IPC: -

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**Patent- og Varemærkestyrelsen**
Økonomi- og Erhvervsministeriet

29 December 2003

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**PATENT- OG VAREMÆRKESTYRELSEN**

**Title: Detergent composition**

The present invention relates to a detergent composition comprising an endo-glucanase that provides a cleaning and anti-redeposition effect. The invention also relates to a detergent composition comprising a combination of an endo-glucanase and other enzymes. One aspect of the invention relates to a process of washing a fabric.

## BACKGROUND OF THE INVENTION

Not all kinds of laundry soils can be satisfactorily removed with conventional detergents. Problem soils include particulate soils such as clays and carbon.

To overcome such problem soils detergents may be added an anti-redeposition agent. An anti-redeposition agent is an agent whereby the above mentioned problem soils detached from the fabrics can be kept dissolved or suspended in the wash liquor in such a way that it is not deposited on the cleaned fabric.

Typical anti-redeposition agents used in detergents include water-soluble, generally organic colloids, including for example the water-soluble salts of polymeric carboxylic acids, glue, gelatine, salts of ether carboxylic acids or ether sulfonic acids of starch or cellulose or salts of sulfuric acid esters of cellulose or starch. Water-soluble polyamides containing acidic groups are also used as anti-redeposition agent. Soluble starch preparations and other starch products than those mentioned above, for example partly hydrolyzed starch, may also be used. Sodium carboxymethyl cellulose, methyl cellulose, methyl hydroxyethyl cellulose and mixtures thereof are preferably used.

To obtain both an anti-redeposition effect and a washing effect it has been suggested to add a mixture of cellulase, one having anti-redeposition effect and one having cleaning effect.

EP patent no. 822,973-A relates to a detergent composition comprising a mixture of cellulases. However, there is a need for improved combinations of enzymes having anti-redeposition and other enzymes.

## SUMMARY OF THE INVENTION

The invention relates to a detergent composition comprising an endo-glucanase having anti-redeposition effect. The inventor has found the combination of such endoglucanase give advantages when used for washing fabrics (especially laundry washing).

The inventor also found detergent compositions comprising specific combinations of certain endo-glucanase(s) having anti-redeposition effect and certain cellulase(s) having increased stability towards anionic tensides, such as linear (straight-chain) alkyl benzene sulfonates (often referred to as "LAS"), have advantages compared to the prior art detergent composition concerned in EP patent no. 822,973-A comprising a combination of cellulases.

especially detergent comprising LAS. For instance, a decreased amount of enzyme protein is necessary to obtain the desired cleaning and anti-redeposition effect. This result in improved product economy.

Thus in the first aspect the invention relates to a detergent composition comprising comprising an endo-glucanase, wherein the endo-glucanase is selected from one of

(i) the endo-glucanase having the amino acid sequence of position 1 to position 773 of SEQ ID NO: 2;

(ii) an endo-glucanase having a sequence of at least 70% identity to the amino acid sequence of position 1 to position 773 of SEQ ID NO:2; or a fragment thereof that has endo-glucanase activity.

**Identity**

In context of the present invention the degree of identity is determined between two sequences indicating a derivation of the first sequence from the second. The identity is determined by means of the computer program GAP provided in the GCG program package. Thus, Gap GCGv8 is used with the following default parameters: GAP creation penalty of 3.0 and GAP extension penalty of 0.1, the default scoring matrix, for protein sequences. GAP uses the method of Needleman/Wunsch/Sellers to make alignments.

In an embodiment the above endoglucanase is combined with other specific enzymes.

In a second aspect the invention relates to a detergent composition comprising anionic tensides and a combination of an endo-glucanase as defined above and a fungal cellulase, wherein both enzymes are stable in the presence of anionic tensides.

In an aspect the invention relates to a process of using a detergent composition of the invention.

**DETAILED DESCRIPTION OF THE INVENTION**

The invention relates to a detergent composition comprising an endo-glucanase having anti-redeposition effect.

<u>The Endoglucanase with anti-redeposition effect</u>

The endo-glucanase having anti-redeposition effect is according to the invention selected from one of

(i) the endo-glucanase having the amino acid sequence of position 1 to position 773 of SEQ ID NO: 2;

(ii) an endo-glucanase having a sequence of at least 70% identity to the amino acid sequence of position 1 to position 773 of SEQ ID NO:2; or a fragment thereof that has endo-glucanase activity.

Endoglucanase used in detergent compositions are also more stable in detergent compositions comprising anionic tensides, such as LAS, than the commercial available 43 kD cellulase derived from *Humicola insolens* DSM 1800 disclosed in WO 91/17243 (SEQ ID NO: 2 and hereby incorporated by reference). Said 43 kD cellulase is available from Novozymes A/S (Denmark) as CAREZYME™.

In a preferred embodiment the endo-glucanase is derived from *Bacillus* sp., DSM 12648 and also shown in SEQ ID NO: 2 herein or a sequence being 70% identical thereto, such as the endoglucanase derived from *Bacillus* sp. KSMS237 deposited as FERM P-16067 and shown in position 1 to 824 of SEQ ID NO: 1 of JP 2000210081 A (hereby incorporated by reference).

Detergent composition comprising anionic tensides

In a preferred embodiment the invention relates to a detergent composition comprising anionic tensides and a combination of an endo-glucanase as defined above and a fungal cellulase, wherein both enzymes are stable in the presence of anionic tensides, such as LAS.

Thus, in a preferred embodiment the invention relates to a detergent composition wherein

(a) the endo-glucanase is selected from one of

(i) the endo-glucanase having the amino acid sequence of position 1 to position 773 of SEQ ID NO. 2;

(i) an endo-glucanase having a sequence of at least 70% identity to the amino acid sequence of position 1 to position 773 of SEQ ID NO:2; or a fragment thereof that has endo-glucanase activity; and

(b) the cellulase is selected from one of

(i) the cellulase having the amino acid sequence of position 1 to position 299 of SEQ ID NO: 9 or

(i) a cellulase having a sequence of at least 70% identity to the amino acid sequence of position 1 to position 299 of SEQ ID NO:4, or a fragment thereof that has cellulase activity.

In a preferred embodiment the cellulase is a *Thielavia terrestris* cellulase, preferably the cellulase disclosed in SEQ ID NO: 9 in WO 96/29397 and SEQ ID NO: 4 herein or an enzyme with at least 70% identity thereto In preferred embodiment cellulase is the *Thielavia terrestris* variant disclosed in Example 1 of WO 98/12307.

Detergent composition of the invention

The detergent compositions according to the present invention comprise a surfactant system, wherein the surfactant can be selected from nonionic and/or anionic and/or cationic and/or ampholytic and/or zwitterionic and/or semi-polar surfactants.

The surfactant is typically present at a level from 0.1% to 60% by weight.

The surfactant is preferably formulated to be compatible with enzyme components present in the composition. In liquid or gel compositions the surfactant is most preferably formulated in such a way that it promotes, or at least does not degrade, the stability of any enzyme in these compositions.

Preferred systems to be used according to the present invention comprise as a surfactant one or more of the nonionic and/or anionic surfactants described herein.

Polyethylene, polypropylene, and polybutylene oxide condensates of alkyl phenols are suitable for use as the nonionic surfactant of the surfactant systems of the present invention, with the polyethylene oxide condensates being preferred. These compounds include the condensation products of alkyl phenols having an alkyl group containing from about 6 to about 14 carbon atoms, preferably from about 8 to about 14 carbon atoms, in either a straight chain or branched-chain configuration with the alkylene oxide. In a preferred embodiment, the ethylene oxide is present in an amount equal to from about 2 to about 25 moles, more preferably from about 3 to about 15 moles, of ethylene oxide per mole of alkyl phenol. Commercially available nonionic surfactants of this type include Igepal™ CO-630, marketed by the GAF Corporation, and Triton™ X-45, X-114, X-100 and X-102, all marketed by the Rohm & Haas Company. These surfactants are commonly referred to as alkylphenol alkoxylates (e.g., alkyl phenol ethoxylates).

The condensation products of primary and secondary aliphatic alcohols with about 1 to about 25 moles of ethylene oxide are suitable for use as the nonionic surfactant of the nonionic surfactant systems of the present invention. The alkyl chain of the aliphatic alcohol can either be straight or branched, primary or secondary, and generally contains from about 8 to about 22 carbon atoms. Preferred are the condensation products of alcohols having an alkyl group containing from about 8 to about 20 carbon atoms, more preferably from about 10 to about 18 carbon atoms, with from about 2 to about 10 moles of ethylene oxide per mole of alcohol. About 2 to about 7 moles of ethylene oxide and most preferably from 2 to 5 moles of ethylene oxide per mole of alcohol are present in said condensation products. Examples of commercially available nonionic surfactants of this type include Tergitol™ 15-S-9 (The condensation product of $C_{11}$-$C_{15}$ linear alcohol with 9 moles ethylene oxide), Tergitol™ 24-L-6 NMW (the condensation product of $C_{12}$-$C_{14}$ primary alcohol with 6 moles ethylene oxide with a narrow molecular weight distribution), both marketed by Union Carbide Corporation; Neodol™ 45-9 (the condensation product of $C_{14}$-$C_{15}$ linear alcohol with 9 moles of ethylene oxide), Neodol™ 23-3 (the condensation product of $C_{12}$-$C_{13}$ linear alcohol with 3.0 moles of ethylene

oxide), Neodol™ 45-7 (the condensation product of $C_{14}$-$C_{15}$ linear alcohol with 7 moles of ethylene oxide), Neodol™ 45-5 (the condensation product of $C_{14}$-$C_{15}$ linear alcohol with 5 moles of ethylene oxide) marketed by Shell Chemical Company, Kyro™ EOB (the condensation product of $C_{13}$-$C_{15}$ alcohol with 9 moles ethylene oxide), marketed by The Procter & Gamble Company, and Genapol LA 050 (the condensation product of $C_{12}$-$C_{14}$ alcohol with 5 moles of ethylene oxide) marketed by Hoechst. Preferred range of HLB in these products is from 8-11 and most preferred from 8-10.

Also useful as the nonionic surfactant of the surfactant systems of the present invention are alkylpolysaccharides disclosed in US 4,565,647, having a hydrophobic group containing from about 6 to about 30 carbon atoms, preferably from about 10 to about 16 carbon atoms and a polysaccharide, e.g. a polyglycoside, hydrophilic group containing from about 1.3 to about 10, preferably from about 1.3 to about 3, most preferably from about 1.3 to about 2.7 saccharide units. Any reducing saccharide containing 5 or 6 carbon atoms can be used, e.g., glucose, galactose and galactosyl moieties can be substituted for the glucosyl moieties (optionally the hydrophobic group is attached at the 2-, 3-, 4-, etc. positions thus giving a glucose or galactose as opposed to a glucoside or galactoside). The intersaccharide bonds can be, e.g., between the one position of the additional saccharide units and the 2-, 3-, 4-, and/or 6- positions on the preceding saccharide units.

The preferred alkylpolyglycosides have the formula

$$R^2O(C_nH_{2n}O)_t(\text{glycosyl})_x$$

wherein $R^2$ is selected from the group consisting of alkyl, alkylphenyl, hydroxyalkyl, hydroxyalkylphenyl, and mixtures thereof in which the alkyl groups contain from about 10 to about 18, preferably from about 12 to about 14, carbon atoms; n is 2 or 3, preferably 2; t is from 0 to about 10, pre-ferably 0; and x is from about 1.3 to about 10, preferably from about 1.3 to about 3, most preferably from about 1.3 to about 2.7. The glycosyl is preferably derived from glucose. To prepare these compounds, the alcohol or alkylpolyethoxy alcohol is formed first and then reacted with glucose, or a source of glucose, to form the glucoside (attachment at the 1-position). The additional glycosyl units can then be attached between their 1-position and the preceding glycosyl units 2-, 3-, 4-, and/or 6-position, preferably predominantly the 2-position.

The condensation products of ethylene oxide with a hydrophobic base formed by the condensation of propylene oxide with propylene glycol are also suitable for use as the additional nonionic surfactant systems of the present invention. The hydrophobic portion of these compounds will preferably have a molecular weight from about 1500 to about 1800 and will exhibit water insolubility. The addition of polyoxyethylene moieties to this hydrophobic portion tends to increase the water solubility of the molecule as a whole, and the liquid character of the product is retained up to the point where the polyoxyethylene content is about

50% of the total weight of the condensation product, which corresponds to condensation with up to about 40 moles of ethylene oxide Examples of compounds of this type include certain of the commercially available Pluronic™ surfactants, marketed by BASF.

Also suitable for use as the nonionic surfactant of the nonionic surfactant system of the present invention, are the condensation products of ethylene oxide with the product resulting from the reaction of propylene oxide and ethylenediamine. The hydrophobic moiety of these products consists of the reaction product of ethylenediamine and excess propylene oxide, and generally has a molecular weight of from about 2500 to about 3000. This hydrophobic moiety is condensed with ethylene oxide to the extent that the condensation product contains from about 40% to about 80% by weight of polyoxyethylene and has a molecular weight of from about 5,000 to about 11,000. Examples of this type of nonionic surfactant include certain of the commercially available Tetronic™ compounds, marketed by BASF.

Preferred for use as the nonionic surfactant of the surfactant systems of the present invention are polyethylene oxide condensates of alkyl phenols, condensation products of primary and secondary aliphatic alcohols with from about 1 to about 25 moles of ethyleneoxide, alkylpolysaccharides, and mixtures hereof. Most preferred are $C_8$-$C_{14}$ alkyl phenol ethoxylates having from 3 to 15 ethoxy groups and $C_8$-$C_{18}$ alcohol ethoxylates (preferably $C_{10}$ avg.) having from 2 to 10 ethoxy groups, and mixtures thereof.

Highly preferred nonionic surfactants are polyhydroxy fatty acid amide surfactants of the formula

$$R^2 - C - N - Z,$$
$$\quad\quad \| \quad\; | \quad\; |$$
$$\quad\quad O \quad R^1$$

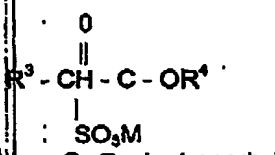
wherein $R^1$ is H, or $R^1$ is $C_{1-4}$ hydrocarbyl, 2-hydroxyethyl, 2-hydroxypropyl or a mixture thereof, $R^2$ is $C_{5-31}$ hydrocarbyl, and Z is a polyhydroxyhydrocarbyl having a linear hydrocarbyl chain with at least 3 hydroxyls directly connected to the chain, or an alkoxylated derivative thereof. Preferably, $R^1$ is methyl, $R^2$ is straight $C_{11-15}$ alkyl or $C_{16-18}$ alkyl or alkenyl chain such as coconut alkyl or mixtures thereof, and Z is derived from a reducing sugar such as glucose, fructose, maltose or lactose, in a reductive amination reaction.

Highly preferred anionic surfactants include alkyl alkoxylated sulfate surfactants. Examples hereof are water soluble salts or acids of the formula $RO(A)_mSO3M$ wherein R is an unsubstituted $C_{10}$-$C_{24}$ alkyl or hydroxyalkyl group having a $C_{10}$-$C_{24}$ alkyl component, preferably a $C_{12}$-$C_{20}$ alkyl or hydro-xyalkyl, more preferably $C_{12}$-$C_{18}$ alkyl or hydroxyalkyl, A is an ethoxy or propoxy unit, m is greater than zero, typically between about 0.5 and about 6, more preferably between about 0.6 and about 3, and M is H or a cation which can be, for example, a metal cation (e.g., sodium, potassium, lithium, calcium, magnesium, etc.), ammonium or substituted-ammonium cation. Alkyl ethoxylated sulfates as well as alkyl

propoxylated sulfates are contemplated herein. Specific examples of substituted ammonium cations include methyl-, dimethyl, trimethyl-ammonium cations and quaternary ammonium cations such as tetramethyl-ammonium and dimethyl piperdinium cations and those derived from alkylamines such as ethylamine, diethylamine, triethylamine, mixtures thereof, and the like. Exemplary surfactants are $C_{12}$-$C_{18}$ alkyl polyethoxylate (1.0) sulfate ($C_{12}$-$C_{18}$E(1.0)M), $C_{12}$-$C_{18}$ alkyl polyethoxylate (2.25) sulfate ($C_{12}$-$C_{18}$(2.25)M, and $C_{12}$-$C_{18}$ alkyl polyethoxylate (3.0) sulfate ($C_{12}$-$C_{18}$E(3.0)M), and $C_{12}$-$C_{18}$ alkyl polyethoxylate (4.0) sulfate ($C_{12}$-$C_{18}$E(4.0)M), wherein M is conveniently selected from sodium and potassium.

Suitable anionic surfactants to be used are alkyl ester sulfonate surfactants including linear esters of $C_8$-$C_{20}$ carboxylic acids (i.e., fatty acids) which are sulfonated with gaseous $SO_3$ according to "The Journal of the American Oil Chemists Society", 52 (1975), pp. 323-329. Suitable starting materials would include natural fatty substances as derived from tallow, palm oil, etc.

The preferred alkyl ester sulfonate surfactant, especially for laundry applications, comprises alkyl ester sulfonate surfactants of the structural formula:



wherein $R^3$ is a $C_8$-$C_{20}$ hydrocarbyl, preferably an alkyl, or combination thereof, $R^4$ is a $C_1$-$C_6$ hydrocarbyl, preferably an alkyl, or combination thereof, and M is a cation which forms a water soluble salt with the alkyl ester sulfonate. Suitable salt-forming cations include metals such as sodium, potassium, and lithium, and substituted or unsubstituted ammonium cations, such as monoethanolamine, diethonolamine, and triethanolamine. Preferably, $R^3$ is $C_{10}$-$C_{16}$ alkyl, and $R^4$ is methyl, ethyl or isopropyl. Especially preferred are the methyl ester sulfonates wherein $R^3$ is $C_{10}$-$C_{16}$ alkyl.

Other suitable anionic surfactants include the alkyl sulfate surfactants which are water soluble salts or acids of the formula $ROSO_3M$ wherein R preferably is a $C_{10}$-$C_{24}$ hydrocarbyl, preferably an alkyl or hydroxyalkyl having a $C_{10}$-$C_{20}$ alkyl component, more preferably a $C_{12}$-$C_{18}$ alkyl or hydroxyalkyl, and M is H or a cation, e.g., an alkali metal cation (e.g. sodium, potassium, lithium), or ammonium or substituted ammonium (e.g. methyl-, dimethyl-, and trimethyl ammonium cations and quaternary ammonium cations such as tetramethyl-ammonium and dimethyl piperdinium cations and quaternary ammonium cations derived from alkylamines such as ethylamine, diethylamine, triethylamine, and mixtures thereof, and the like). Typically, alkyl chains of $C_{12}$-$C_{16}$ are preferred for lower wash temperatures (e.g. below about 50°C) and $C_{16}$-$C_{18}$ alkyl chains are preferred for higher wash temperatures (e.g. above about 50°C).

Other anionic surfactants useful for detersive purposes can also be included in the laundry detergent compositions of the present invention. Theses can include salts (including, for example, sodium, potassium, ammonium, and substituted ammonium salts such as mono- di- and triethanolamine salts) of soap, $C_8$-$C_{22}$ primary or secondary alkanesulfonates, $C_8$-$C_{24}$ olefinsulfonates, sulfonated polycarboxylic acids prepared by sulfonation of the pyrolyzed product of alkaline earth metal citrates, e.g., as described in British patent specification No. 1,082,179, $C_8$-$C_{24}$ alkylpolyglycolethersulfates (containing up to 10 moles of ethylene oxide); alkyl glycerol sulfonates, fatty acyl glycerol sulfonates, fatty oleyl glycerol sulfates, alkyl phenol ethylene oxide ether sulfates, paraffin sulfonates, alkyl phosphates, isethionates such as the acyl isethionates, N-acyl taurates, alkyl succinamates and sulfosuccinates, monoesters of sulfosuccinates (especially saturated and unsaturated $C_{12}$-$C_{18}$ monoesters) and diesters of sulfosuccinates (especially saturated and unsaturated $C_6$-$C_{12}$ diesters), acyl sarcosinates, sulfates of alkylpolysaccharides such as the sulfates of alkylpolyglucoside (the nonionic nonsulfated compounds being described below), branched primary alkyl sulfates, and alkyl polyethoxy carboxylates such as those of the formula $RO(CH_2CH_2O)_k$-$CH_2COO$-M+ wherein R is a $C_8$-$C_{22}$ alkyl, k is an integer from 1 to 10, and M is a soluble salt forming cation. Resin acids and hydrogenated resin acids are also suitable, such as rosin, hydrogenated rosin, and resin acids and hydrogenated resin acids present in or derived from tall oil.

Alkylbenzene sulfonates are highly preferred. Especially preferred are linear (straight-chain) alkyl benzene sulfonates (LAS) wherein the alkyl group preferably contains from 10 to 18 carbon atoms.

Further examples are described in "Surface Active Agents and Detergents" (Vol. I and II by Schwartz, Perrry and Berch). A variety of such surfactants are also generally disclosed in US 3,929,678, (Column 23, line 58 through Column 29, line 23, herein incorporated by reference).

When included therein, the laundry detergent compositions of the present invention typically comprise from about 1% to about 40%, preferably from about 3% to about 20% by weight of such anionic surfactants.

The laundry detergent compositions of the present invention may also contain cationic, ampholytic, zwitterionic, and semi-polar surfactants, as well as the nonionic and/or anionic surfactants other than those already described herein.

Cationic detersive surfactants suitable for use in the laundry detergent compositions of the present invention are those having one long-chain hydrocarbyl group. Examples of such cationic surfactants include the ammonium surfactants such as alkyltrimethylammonium halogenides, and those surfactants having the formula:

$$[R^2(OR^3)_y][R^4(OR^3)_y]_2R^5N+X-$$

wherein $R^2$ is an alkyl or alkyl benzyl group having from about 8 to about 18 carbon atoms in the alkyl chain, each $R^3$ is selected form the group consisting of $-CH_2CH_2-$, $-CH_2CH(CH_3)-$, $-CH_2CH(CH_2OH)-$, $-CH_2CH_2CH_2-$, and mixtures thereof; each $R^4$ is selected from the group consisting of $C_1$-$C_4$ alkyl, $C_1$-$C_4$ hydroxyalkyl, benzyl ring structures formed by joining the two $R^4$ groups, $-CH_2CHOHCHOHCOR^6CHOHCH_2OH$, wherein $R^6$ is any hexose or hexose polymer having a molecular weight less than about 1000, and hydrogen when y is not 0; $R^5$ is the same as $R^4$ or is an alkyl chain,wherein the total number of carbon atoms or $R^2$ plus $R^5$ is not more than about 18; each y is from 0 to about 10,and the sum of the y values is from 0 to about 15; and X is any compatible anion.

Highly preferred cationic surfactants are the water soluble quaternary ammonium compounds useful in the present composition having the formula:

$$R_1R_2R_3R_4N^+X^- \quad (i)$$

wherein $R_1$ is $C_8$-$C_{16}$ alkyl, each of $R_2$, $R_3$ and $R_4$ is independently $C_1$-$C_4$ alkyl, $C_1$-$C_4$ hydroxy alkyl, benzyl, and $-(C_2H_{40})_xH$ where x has a value from 2 to 5, and X is an anion. Not more than one of $R_2$, $R_3$ or $R_4$ should be benzyl.

The preferred alkyl chain length for $R_1$ is $C_{12}$-$C_{15}$, particularly where the alkyl group is a mixture of chain lengths derived from coconut or palm kernel fat or is derived synthetically by olefin build up or OXO alcohols synthesis.

Preferred groups for $R_2R_3$ and $R_4$ are methyl and hydroxyethyl groups and the anion X may be selected from halide, methosulphate, acetate and phosphate ions.

Examples of suitable quaternary ammonium compounds of formulae (i) for use herein are:

coconut trimethyl ammonium chloride or bromide;

coconut methyl dihydroxyethyl ammonium chloride or bromide;

decyl triethyl ammonium chloride;

decyl dimethyl hydroxyethyl ammonium chloride or bromide;

$C_{12-15}$ dimethyl hydroxyethyl ammonium chloride or bromide;

coconut dimethyl hydroxyethyl ammonium chloride or bromide;

myristyl trimethyl ammonium methyl sulphate;

lauryl dimethyl benzyl ammonium chloride or bromide;

lauryl dimethyl $(ethenoxy)_4$ ammonium chloride or bromide;

choline esters (compounds of formula (i) wherein $R_1$ is $CH_2$-$CH_2$-O-C-$C_{12-14}$ alkyl and $R_2R_3R_4$ are methyl).



di-alkyl imidazolines [compounds of formula (i)].

Other cationic surfactants useful herein are also described in US 4,228,044 and in EP 000 224.

When included therein, the laundry detergent compositions of the present invention typically comprise from 0.2% to about 25%, preferably from about 1% to about 8% by weight of such cationic surfactants.

Ampholytic surfactants are also suitable for use in the laundry detergent compositions of the present invention. These surfactants can be broadly described as aliphatic derivatives of secondary or tertiary amines, or aliphatic derivatives of heterocyclic secondary and tertiary amines in which the aliphatic radical can be straight- or branched-chain. One of the aliphatic substituents contains at least about 8 carbon atoms, typically from about 8 to about 18 carbon atoms, and at least one contains an anionic water-solubilizing group, e.g. carboxy, sulfonate, sulfate. See US 3,929,678 (column 19, lines 18-35) for examples of ampholytic surfactants.

When included therein, the laundry detergent compositions of the present invention typically comprise from 0.2% to about 15%, preferably from about 1% to about 10% by weight of such ampholytic surfactants.

Zwitterionic surfactants are also suitable for use in laundry detergent compositions. These surfactants can be broadly described as derivatives of secondary and tertiary amines, derivatives of heterocyclic secondary and tertiary amines, or derivatives of quaternary ammonium, quaternary phosphonium or tertiary sulfonium compounds. See US 3,929,678 (column 19, line 38 through column 22, line 48) for examples of zwitterionic surfactants.

When included therein, the laundry detergent compositions of the present invention typically comprise from 0.2% to about 15%, preferably from about 1% to about 10% by weight of such zwitterionic surfactants.

Semi-polar nonionic surfactants are a special category of nonionic surfactants which include water-soluble amine oxides containing one alkyl moiety of from about 10 to about 18 carbon atoms and 2 moieties selected from the group consisting of alkyl groups and hydroxyalkyl groups containing from about 1 to about 3 carbon atoms; watersoluble phosphine oxides containing one alkyl moiety of from about 10 to about 18 carbon atoms and 2 moieties selected from the group consisting of alkyl groups and hydroxyalkyl groups containing from about 1 to about 3 carbon atoms; and water-soluble sulfoxides containing one alkyl moiety from about 10 to about 18 carbon atoms and a moiety selected from the group consisting of alkyl and hydroxyalkyl moieties of from about 1 to about 3 carbon atoms.

Semi-polar nonionic detergent surfactants include the amine oxide surfactants having the formula:

$$R^3(OR^4)xN(R^5)2 \rightarrow O$$

wherein $R^3$ is an alkyl, hydroxyalkyl, or alkyl phenyl group or mixtures thereof containing from about 8 to about 22 carbon atoms; $R^4$ is an alkylene or hydroxyalkylene group containing from

about 2 to about 3 carbon atoms or mixtures thereof; x is from 0 to about 3: and each $R^5$ is an alkyl or hydroxyalkyl group containing from about 1 to about 3 carbon atoms or a polyethylene oxide group containing from about 1 to about 3 ethylene oxide groups. The $R^5$ groups can be attached to each other, e g., through an oxygen or nitrogen atom, to form a ring structure.

These amine oxide surfactants in particular include $C_{10}$-$C_{18}$ alkyl dimethyl amine oxides and $C_8$-$C_{12}$ alkoxy ethyl dihydroxy ethyl amine oxides.

When included therein, the laundry detergent compositions of the present invention typically comprise from 0.2% to about 15%, preferably from about 1% to about 10% by weight of such semi-polar nonionic surfactants.

Builder system

The compositions according to the present invention may further comprise a builder system. Any conventional builder system is suitable for use herein including aluminosilicate materials, silicates, polycarboxylates and fatty acids, materials such as ethylenediamine tetraacetate, metal ion sequestrants such as aminopolyphosphonates, particularly ethylenediamine tetramethylene phosphonic acid and diethylene triamine pentamethylenephosphonic acid. Though less preferred for obvious environmental reasons, phosphate builders can also be used herein.

Suitable builders can be an inorganic ion exchange material, commonly an inorganic hydrated aluminosilicate material, more particularly a hydrated synthetic zeolite such as hydrated zeolite A, X, B, HS or MAP.

Another suitable inorganic builder material is layered silicate, e.g. SKS-6 (Hoechst). SKS-6 is a crystalline layered silicate consisting of sodium silicate ($Na_2Si_2O_5$).

Suitable polycarboxylates containing one carboxy group include lactic acid, glycolic acid and ether derivatives thereof as disclosed in Belgian Patent Nos. 831,368, 821,369 and 821,370. Polycarboxylates containing two carboxy groups include the water-soluble salts of succinic acid, malonic acid, (ethylenedioxy) diacetic acid, maleic acid, diglycollic acid, tartaric acid, tartronic acid and fumaric acid, as well as the ether carboxylates described in DE 2,446,686, and 2,446,487, US 3,935,257 and the sulfinyl carboxylates described in Belgian Patent No. 840,623. Polycarboxylates containing three carboxy groups include, in particular, water-soluble citrates, aconitrates and citraconates as well as succinate derivatives such as the carboxymethyloxysuccinates described in British Patent No. 1,379,241, lactoxysuccinates described in Netherlands Application 7205873, and the oxypolycarboxylate materials such as 2-oxa-1,1,3-propane tricarboxylates described in British Patent No. 1,387,447.

Polycarboxylates containing four carboxy groups include oxydisuccinates disclosed in British Patent No. 1,261,829, 1,1,2,2,-ethane tetracarboxylates, 1,1,3,3-propane tetracarboxylates containing sulfo substituents include the sulfosuccinate derivatives

disclosed in British Patent Nos. 1,398,421 and 1,398,422 and in US 3,936,448, and the sulfonated pyrolysed citrates described in British Patent No. 1,082,179, while polycarboxylates containing phosphone substituents are disclosed in British Patent No. 1,439,000.

Alicyclic and heterocyclic polycarboxylates include cyclopentane-cis,cis-cis-tetracarboxylates, cyclopentadienide pentacarboxylates, 2,3,4,5-tetrahydro-furan - cis, cis, cis-tetracarboxylates, 2,5-tetrahydro-furan-cis, discarboxylates, 2,2,5,5,-tetrahydrofuran - tetracarboxylates, 1,2,3,4,5,6-hexane - hexacarboxylates and carboxymethyl derivatives of polyhydric alcohols such as sorbitol, mannitol and xylitol. Aromatic polycarboxylates include mellitic acid, pyromellitic acid and the phthalic acid derivatives disclosed in British Patent No. 1,425,343.

Of the above, the preferred polycarboxylates are hydroxy-carboxylates containing up to three carboxy groups per molecule, more particularly citrates.

Preferred builder systems for use in the present compositions include a mixture of a water-insoluble aluminosilicate builder such as zeolite A or of a layered silicate (SKS-6), and a water-soluble carboxylate chelating agent such as citric acid.

A suitable chelant for inclusion in the detergent composi-ions in accordance with the invention is ethylenediamine-N,N'-disuccinic acid (EDDS) or the alkali metal, alkaline earth metal, ammonium, or substituted ammonium salts thereof, or mixtures thereof. Preferred EDDS compounds are the free acid form and the sodium or magnesium salt thereof. Examples of such preferred sodium salts of EDDS include $Na_2EDDS$ and $Na_4EDDS$. Examples of such preferred magnesium salts of EDDS include MgEDDS and $Mg_2EDDS$. The magnesium salts are the most preferred for inclusion in compositions in accordance with the invention.

Preferred builder systems include a mixture of a water-insoluble aluminosilicate builder such as zeolite A, and a water soluble carboxylate chelating agent such as citric acid.

Other builder materials that can form part of the builder system for use in granular compositions include inorganic materials such as alkali metal carbonates, bicarbonates, silicates, and organic materials such as the organic phosphonates, amino polyalkylene phosphonates and amino polycarboxylates.

Other suitable water-soluble organic salts are the homo- or co-polymeric acids or their salts, in which the polycarboxylic acid comprises at least two carboxyl radicals separated form each other by not more than two carbon atoms.

Polymers of this type are disclosed in GB-A-1,596,756. Examples of such salts are polyacrylates of MW 2000-5000 and their copolymers with maleic anhydride, such copolymers having a molecular weight of from 20,000 to 70,000, especially about 40,000.

Detergency builder salts are normally included in amounts of from 5% to 80% by weight of the composition. Preferred levels of builder for liquid detergents are from 5% to 30%.

Enzymes

A detergent compositions of the invention may in an embodiment of the invention besides the endo-glucanase having anti-redeposition effect as defined above, comprise other enzyme(s) which provides cleaning performance and/or fabric care benefits.

Such enzymes include certain proteases, lipases, cutinases, cellulases, amylases, peroxidases, oxidases (e.g. laccases), mannanase and pectate lyase.

Proteases: Any protease suitable for use in alkaline solutions can be used. Suitable proteases include those of animal, vegetable or microbial origin. Microbial origin is preferred. Chemically or genetically modified mutants are included. The protease may be a serine protease, preferably an alkaline microbial protease or a trypsin-like protease. Examples of alkaline proteases are subtilisins, especially those derived from *Bacillus*, e.g., subtilisin Novo, subtilisin Carlsberg, subtilisin 309, subtilisin 147 and subtilisin 168 (described in WO 89/06279). Examples of trypsin-like proteases are trypsin (e g. of porcine or bovine origin) and the Fusarium protease described in WO 89/06270.

Preferred commercially available protease enzymes include those sold under the trade names Everlase™, Kannase™, Alcalase™, Savinase™, Primase™, Durazym™, and Esperase™ by Novozymes A/S (Denmark), those sold under the tradename Maxatase, Maxacal, Maxapem, Properase, Purafect and Purafect OXP by Genencor International, and those sold under the tradename Opticlean and Optimase by Solvay Enzymes. Protease enzymes may be incorporated into the compositions in accordance with the invention at a level of from 0.00001% to 2% of enzyme protein by weight of the composition, preferably at a level of from 0.0001% to 1% of enzyme protein by weight of the composition, more preferably at a level of from 0.001% to 0.5% of enzyme protein by weight of the composition, even more preferably at a level of from 0 01% to 0.2% of enzyme protein by weight of the composition.

Lipases: Any lipase suitable for use in alkaline solutions can be used. Suitable lipases include those of bacterial or fungal origin. Chemically or genetically modified mutants are included

Examples of useful lipases include a Humicola lanuginosa lipase, e.g., as described in EP 258 068 and EP 305 216, a Rhizomucor miehei lipase, e.g., as described in EP 238 023, a Candida lipase, such as a C. antarctica lipase, e.g., the C. antarctica lipase A or B described in EP 214 761, a Pseudomonas lipase such as a P. alcaligenes and P. pseudoalcaligenes lipase, e.g., as described in EP 218 272, a P. cepacia lipase, e.g., as

described in EP 331 376, a P. stutzeri lipase, e.g., as disclosed in GB 1,372,034, a P. fluorescens lipase, a Bacillus lipase, e.g., a B. subtilis lipase (Dartois et al., (1993), Biochemica et Biophysica acta 1131, 253-260), a B. stearothermophilus lipase (JP 64/744992) and a B. pumilus lipase (WO 91/16422).

Furthermore, a number of cloned lipases may be useful, including the *Penicillium camemberti* lipase described by Yamaguchi et al., (1991), Gene 103, 61-67), the *Geotricum candidum* lipase (Schimada, Y. et al., (1989), J. Biochem., 106, 383-388), and various *Rhizopus* lipases such as a *R. delemar* lipase (Hass, M.J et al., (1991), Gene 109, 117-113), a *R. niveus* lipase (Kugimiya et al., (1992), Biosci. Biotech. Biochem. 56, 716-719) and a R. oryzae lipase.

Other types of lipolytic enzymes such as cutinases may also be useful, e.g., a cutinase derived from *Pseudomonas mendocina* as described in WO 88/09367, or a cutinase derived from *Fusarium solani pisi* (e.g. described in WO 90/09446).

In a preferred embodiment the lipase is a variant of *Humicola lanuginosa* DSM 4109 as described in WO 00/60063. Especially preferred are the variants disclosed in the Example in WO 00/60063 with improved first wash performance., i.e., T231R+N233R; G91A +D96W +E99K +G263Q +L264A+I265T+G266D+T267A+L269N+270AGGFSWRRYRSAESVDKRATMTDAELEKKLN SYVQMDKEYVKNNQARS; R209P +T231R +N233R ; N33Q +D96S +T231R +N233R +Q249R; E99N +N101S +T231R +N233R +Q249R; E99N +N101S +T231R +N233R +Q249R.

Especially suitable lipases are lipases such as M1 Lipase™, Luma fast™ and Lipomax™ (Genencor), Lipolase™ and Lipolase Ultra™ Lipex™ (Novozymes A/S), and Lipase P "Amano" (Amano Pharmaceutical Co. Ltd.).

The lipases are normally incorporated in the detergent composition at a level of from 0.00001% to 2% of enzyme protein by weight of the composition, preferably at a level of from 0.0001% to 1% of enzyme protein by weight of the composition, more preferably at a level of from 0.001% to 0.5% of enzyme protein by weight of the composition, even more preferably at a level of from 0.01% to 0.2% of enzyme protein by weight of the composition.

Amylases: Any amylase (alpha and/or beta) suitable for use in alkaline solutions can be used. Suitable amylases include those of bacterial or fungal origin. Chemically or genetically modified mutants are included. Amylases include, for example, alpha-amylases obtained from a special strain of *B. licheniformis*, described in more detail in GB 1,296,839. Commercially available amylases are Natalase™, Termamyl™ Ultra, Duramyl™, Termamyl™, Fungamyl™ and BAN™ (available from Novozymes A/S) and Rapidase™ and Maxamyl P™ (available from Genencor).

In a preferred embodiment the alpha-amylase is derived from *Bacillus* sp. strains NCIB 12289, NCIB 12512, NCIB 12513 and DSM 9375. Especially preferred are the alpha-amylases shown in SEQ ID NOS 1 and 2 of WO 95/26397.

In another preferred embodiment the alpha-amylase is the AA560 alpha-amylase derived from *Bacillus* sp. DSM 12649 disclosed as SEQ ID NO: 2 in WO 00/60060 (hereby incorporated by reference). Especially preferred are variants of the AA560 alpha-amylase, including the AA560 variant disclosed in Example 7 and 8 (hereby incorporated by reference.

The amylases are normally incorporated in the detergent composition at a level of from 0.00001% to 2% of enzyme protein by weight of the composition, preferably at a level of from 0.0001% to 1% of enzyme protein by weight of the composition, more preferably at a level of from 0.001% to 0.5% of enzyme protein by weight of the composition, even more preferably at a level of from 0.01% to 0.2% of enzyme protein by weight of the composition.

Cellulases: Any cellulase suitable for use in alkaline solutions can be used. Suitable cellulases include those of bacterial or fungal origin. Chemically or genetically modified mutants are included. Suitable cellulases are disclosed in US 4,435,307, which discloses fungal cellulases produced from *Humicola insolens*. Especially suitable cellulases are the cellulases having colour care benefits. Examples of such cellulases are cellulases described in European patent application No. 0 495 257.

In a preferred embodiment the cellulase is a *Thielavia terrestris* cellulase, preferably the cellulase disclosed in SEQ ID NO: 9 in WO 96/29397 and SEQ ID NO: 9 herein or an enzyme with at least 70% identity thereto. In preferred embodiment cellulase is the *Thielavia terrestris* variant disclosed in Example 1 of WO 98/12307.

Commercially available cellulases include CELLUZYME™ produced by a strain of *Humicola insolens* or RENOIZYME™ (Novozymes A/S), and KAC-500(B)™ (Kao Corporation). Cellulases are normally incorporated in the detergent composition at a level of from 0.00001% to 2% of enzyme protein by weight of the composition, preferably at a level of from 0.0001% to 1% of enzyme protein by weight of the composition, more preferably at a level of from 0.001% to 0.5% of enzyme protein by weight of the composition, even more preferably at a level of from 0.01% to 0.2% of enzyme protein by weight of the composition.

Mannases: Any mannanase suitable for use in alkaline solutions can be used. Suitable mannanases include those of bacterial or fungal origin. Chemically or genetically modified mutants are included.

In a preferred embodiment the mannanase is derived from a strain of the genus *Bacillus*, especially *Bacillus* sp. I633 disclosed in positions 31-330 of SEQ ID NO:2 or in SEQ ID

NO: 5 of WO 99/64619 or *Bacillus agaradhaerens*, for example from the type strain DSM 8721.

Pectate lyase: Any pectate lyase suitable for use in alkaline solutions can be used. Suitable pectate lyases include those of bacterial or fungal origin. Chemically or genetically modified mutants are included.

In a preferred embodiment the pectate lyase is derived from a strain of the genus *Bacillus*, especially a strain of *Bacillus substilis*, especially *Bacillus subtilis* DSM14218 disclosed in SEQ ID NO:2 or a variant thereof disclosed in Example 6 of WO 02/092741.

Peroxidases/Oxidases: Peroxidase enzymes are used in combination with hydrogen peroxide or a source thereof (e.g. a percarbonate, perborate or persulfate). Oxidase enzymes are used in combination with oxygen. Both types of enzymes are used for "solution bleaching", i.e. to prevent transfer of a textile dye from a dyed fabric to another fabric when said fabrics are washed together in a wash liquor, preferably together with an enhancing agent as described in e.g. WO 94/12621 and WO 95/01426. Suitable peroxidases/oxidases include those of plant, bacterial or fungal ongin. Chemically or genetically modified mutants are included.

Peroxidase and/or oxidase enzymes are normally incorporated in the detergent composition at a level of from 0.00001% to 2% of enzyme protein by weight of the composition, preferably at a level of from 0.0001% to 1% of enzyme protein by weight of the composition, more preferably at a level of from 0.001% to 0.5% of enzyme protein by weight of the composition, even more preferably at a level of from 0.01% to 0.2% of enzyme protein by weight of the composition.

Mixtures of the above mentioned enzymes are encompassed herein, in particular a mixture of a protease, an amylase, a lipase and/or a cellulase.

The enzyme of the invention, or any other enzyme incorporated in the detergent composition, is normally incorporated in the detergent composition at a level from 0.00001% to 2% of enzyme protein by weight of the composition, preferably at a level from 0.0001% to 1% of enzyme protein by weight of the composition, more preferably at a level from 0.001% to 0.5% of enzyme protein by weight of the composition, even more preferably at a level from 0.01% to 0.2% of enzyme protein by weight of the composition.

Bleaching agents: Additional optional detergent ingredients that can be included in the detergent compositions of the present invention include bleaching agents such as PB1, PB4 and percarbonate with a particle size of 400-800 microns. These bleaching agent components can include one or more oxygen bleaching agents and, depending upon the bleaching agent chosen, one or more bleach activators. When present oxygen bleaching compounds will

typically be present at levels of from about 1% to about 25%. In general, bleaching compounds are optional added components in non-liquid formulations, e.g. granular detergents.

The bleaching agent component for use herein can be any of the bleaching agents useful for detergent compositions including oxygen bleaches as well as others known in the art.

The bleaching agent suitable for the present invention can be an activated or non-activated bleaching agent

One category of oxygen bleaching agent that can be used encompasses percarboxylic acid bleaching agents and salts thereof. Suitable examples of this class of agents include magnesium monoperoxyphthalate hexahydrate, the magnesium salt of meta-chloro perbenzoic acid, 4-nonylamino-4-oxoperoxybutyric acid and diperoxydodecanedioic acid. Such bleaching agents are disclosed in US 4,483,781, US 740,446, EP 0 133 354 and US 4,412,934. Highly preferred bleaching agents also include 6-nonylamino-6-oxoperoxycaproic acid as described in US 4,634,551.

Another category of bleaching agents that can be used encompasses the halogen bleaching agents. Examples of hypohalite bleaching agents, for example, include trichloro isocyanuric acid and the sodium and potassium dichloroisocyanurates and N-chloro and N-bromo alkane sulphonamides. Such materials are normally added at 0.5-10% by weight of the finished product, preferably 1-5% by weight.

The hydrogen peroxide releasing agents can be used in combination with bleach activators such as tetra-acetylethylenediamine (TAED), nonanoyloxybenzenesulfonate (NOBS, described in US 4,412,934), 3,5-trimethyl-hexsanoloxybenzenesulfonate (ISONOBS, described in EP 120 591) or pentaacetylglucose (PAG), which are perhydrolyzed to form a peracid as the active bleaching species, leading to improved bleaching effect. In addition, very suitable are the bleach activators C8(6-octanamido-caproyl) oxybenzene-sulfonate, C9(6-nonanamido caproyl) oxybenzenesulfonate and C10 (6-decanamido caproyl) oxybenzenesulfonate or mixtures thereof. Also suitable activators are acylated citrate esters such as disclosed in European Patent Application No. 91870207.7.

Useful bleaching agents, including peroxyacids and bleaching systems comprising bleach activators and peroxygen bleaching compounds for use in cleaning compositions according to the invention are described in application USSN 08/136,626.

The hydrogen peroxide may also be present by adding an enzymatic system (i.e. an enzyme and a substrate therefore) which is capable of generation of hydrogen peroxide at the beginning or during the washing and/or rinsing process. Such enzymatic systems are disclosed in European Patent Application EP 0 537 381.

Bleaching agents other than oxygen bleaching agents are also known in the art and can be utilized herein. One type of non-oxygen bleaching agent of particular interest includes photoactivated bleaching agents such as the sulfonated zinc and/or aluminium phthalocyanines. These materials can be deposited upon the substrate during the washing process. Upon irradiation with light, in the presence of oxygen, such as by hanging clothes out to dry in the daylight, the sulfonated zinc phthalocyanine is activated and, consequently, the substrate is bleached. Preferred zinc phthalocyanine and a photoactivated bleaching process are described in US 4,033,718. Typically, detergent composition will contain about 0.025% to about 1.25%, by weight, of sulfonated zinc phthalocyanine.

Bleaching agents may also comprise a manganese catalyst. The manganese catalyst may, e.g., be one of the compounds described in "Efficient manganese catalysts for low-temperature bleaching", Nature 369, 1994, pp. 637-639.

Suds suppressors: Another optional ingredient is a suds suppressor, exemplified by silicones, and silica-silicone mixtures. Silicones can generally be represented by alkylated polysiloxane materials, while silica is normally used in finely divided forms exemplified by silica aerogels and xerogels and hydrophobic silicas of various types. Theses materials can be incorporated as particulates, in which the suds suppressor is advantageously releasably incorporated in a water-soluble or waterdispersible, substantially non surface-active detergent impermeable carrier. Alternatively the suds suppressor can be dissolved or dispersed in a liquid carrier and applied by spraying on to one or more of the other components.

A preferred silicone suds controlling agent is disclosed in US 3,933,672. Other particularly useful suds suppressors are the self-emulsifying silicone suds suppressors, described in German Patent Application DTOS 2,646,126. An example of such a compound is DC-544, commercially available form Dow Corning, which is a siloxane-glycol copolymer. Especially preferred suds controlling agent are the suds suppressor system comprising a mixture of silicone oils and 2-alkyl-alkanols Suitable 2-alkyl-alkanols are 2-butyl-octanol which are commercially available under the trade name Isofol 12 R.

Such suds suppressor system are described in European Patent Application EP 0 593 841.

Especially preferred silicone suds controlling agents are described in European Patent Application No. 92201649.8. Said compositions can comprise a silicone/ silica mixture in combination with fumed nonporous silica such as Aerosil$^{R}$.

The suds suppressors described above are normally employed at levels of from 0.001% to 2% by weight of the composition, preferably from 0.01% to 1% by weight.

Other components: Other components used in detergent compositions may be employed such as soil-suspending agents, soil-releasing agents, optical brighteners, abrasives, bactericides, tarnish inhibitors, coloring agents, and/or encapsulated or nonencapsulated perfumes.

Especially suitable encapsulating materials are water soluble capsules which consist of a matrix of polysaccharide and polyhydroxy compounds such as described in GB 1,464,616.

Other suitable water soluble encapsulating materials comprise dextrins derived from ungelatinized starch acid esters of substituted dicarboxylic acids such as described in US 3,455,838. These acid-ester dextrins are, preferably, prepared from such starches as waxy maize, waxy sorghum, sago, tapioca and potato. Suitable examples of said encapsulation materials include N-Lok manufactured by National Starch. The N-Lok encapsulating material consists of a modified maize starch and glucose. The starch is modified by adding monofunctional substituted groups such as octenyl succinic acid anhydride.

Antiredeposition and soil suspension agents suitable herein include cellulose derivatives such as methylcellulose, carboxymethylcellulose and hydroxyethylcellulose, and homo- or co-polymeric polycarboxylic acids or their salts. Polymers of this type include the polyacrylates and maleic anhydride-acrylic acid copolymers previously mentioned as builders, as well as copolymers of maleic anhydride with ethylene, methylvinyl ether or methacrylic acid, the maleic anhydride constituting at least 20 mole percent of the copolymer. These materials are normally used at levels of from 0.5% to 10% by weight, more preferably form 0.75% to 8%, most preferably from 1% to 6% by weight of the composition.

Preferred optical brighteners are anionic in character, examples of which are disodium 4,4'-bis-(2-diethanolamino-4-anilino -s- triazin-6-ylamino)stilbene-2:2' disulphonate, disodium 4,4' - 4'-bis-(2-morpholino-4-anilino-s-triazin-6-ylamino-stilbene-2:2' - disulphonate, disodium 4,4' - bis-(2,4-dianilino-s-triazin-6-ylamino)stilbene-2:2' - disulphonate, monosodium 4',4'' - bis-(2,4-dianilino-s-tri-azin-6 ylamino)stilbene-2-sulphonate, disodium 4,4' -bis-(2-anilino-4-(N-methyl-N-2-hydroxyethylamino)-s-triazin-6-ylamino)stilbene-2,2' - disulphonate, di-sodium 4,4' -bis-(4-phenyl-2,1,3-triazol-2-yl)-stilbene-2,2' disulphonate, di-so-dium 4,4'bis(2-anilino-4-(1-methyl-2-hydroxyethylamino)-s-triazin-6-ylami-no)stilbene-2,2'disulphonate, sodium 2(stilbyl-4''-(naphtho-1',2':4,5)-1,2,3, - triazole-2''-sulphonate and 4,4'-bis(2-sulphostyryl)biphenyl.

Other useful polymeric materials are the polyethylene glycols, particularly those of molecular weight 1000-10000, more particularly 2000 to 8000 and most preferably about 4000. These are used at levels of from 0.20% to 5% more preferably from 0.25% to 2.5% by weight. These polymers and the previously mentioned homo- or co-polymeric polycarboxylate salts are valuable for improving whiteness maintenance, fabric ash deposition,

and cleaning performance on clay, proteinaceous and oxidizable soils in the presence of transition metal impurities.

Soil release agents useful in compositions of the present invention are conventionally copolymers or terpolymers of terephthalic acid with ethylene glycol and/or propylene glycol units in various arrangements. Examples of such polymers are disclosed in US 4,116,885 and 4,711,730 and EP 0 272 033. A particular preferred polymer in accordance with EP 0 272 033 has the formula:

$$(CH_3(PEG)_{43})_{0.75}(POH)_{0.25}[T\text{-}PO)_{2.8}(T\text{-}PEG)_{0.4}]T(POH)_{0.25}((PEG)_{43}CH_3)_{0.75}$$

where PEG is $-(OC_2H_4)O-$, PO is $(OC_3H_6O)$ and T is $(pOOC_6H_4CO)$.

Also very useful are modified polyesters as random copolymers of dimethyl terephthalate, dimethyl sulfoisophthalate, ethylene glycol and 1,2-propanediol, the end groups consisting primarily of sulphobenzoate and secondarily of mono esters of ethylene glycol and/or 1,2-propanediol. The target is to obtain a polymer capped at both end by sulphobenzoate groups, "primarily", in the present context most of said copolymers herein will be endcapped by sulphobenzoate groups. However, some copolymers will be less than fully capped, and therefore their end groups may consist of monoester of ethylene glycol and/or 1,2-propanediol, thereof consist "secondarily" of such species.

The selected polyesters herein contain about 46% by weight of dimethyl terephthalic acid, about 16% by weight of 1,2-propanediol, about 10% by weight ethylene glycol, about 13% by weight of dimethyl sulfobenzoic acid and about 15% by weight of sulfoisophthalic acid, and have a molecular weight of about 3.000. The polyesters and their method of preparation are described in detail in EP 311 342.

Softening agents: Fabric softening agents can also be incorporated into laundry detergent compositions in accordance with the present invention. These agents may be inorganic or organic in type. Inorganic softening agents are exemplified by the smectite clays disclosed in GB-A-1 400898 and in US 5,019,292. Organic fabric softening agents include the water insoluble tertiary amines as disclosed in GB-A1 514 276 and EP 0 011 340 and their combination with mono $C_{12}$-$C_{14}$ quaternary ammonium salts are disclosed in EP-B-0 026 528 and di-long-chain amides as disclosed in EP 0 242 919. Other useful organic ingredients of fabric softening systems include high molecular weight polyethylene oxide materials as disclosed in EP 0 299 575 and 0 313 146.

Levels of smectite clay are normally in the range from 5% to 15%, more preferably from 8% to 12% by weight, with the material being added as a dry mixed component to the

remainder of the formulation. Organic fabric softening agents such as the water-insoluble tertiary amines or dilong chain amide materials are incorporated at levels of from 0.5% to 5% by weight, normally from 1% to 3% by weight whilst the high molecular weight polyethylene oxide materials and the water soluble cationic materials are added at levels of from 0.1% to 2%, normally from 0.15% to 1.5% by weight. These materials are normally added to the spray dried portion of the composition, although in some instances it may be more convenient to add them as a dry mixed particulate, or spray them as molten liquid on to other solid components of the composition.

Polymeric dye-transfer inhibiting agents: The detergent compositions according to the present invention may also comprise from 0.001% to 10%, preferably from 0.01% to 2%, more preferably form 0.05% to 1% by weight of polymeric dye- transfer inhibiting agents. Said polymeric dye-transfer inhibiting agents are normally incorporated into detergent compositions in order to inhibit the transfer of dyes from colored fabrics onto fabrics washed therewith. These polymers have the ability of complexing or adsorbing the fugitive dyes washed out of dyed fabrics before the dyes have the opportunity to become attached to other articles in the wash.

Especially suitable polymeric dye-transfer inhibiting agents are polyamine N-oxide polymers, copolymers of N-vinyl-pyrrolidone and N-vinylimidazole, polyvinylpyrrolidone polymers, polyvinyloxazolidones and polyvinylimidazoles or mixtures thereof.

Addition of such polymers also enhances the performance of the enzymes according the invention.

The detergent composition according to the invention can be in liquid, paste, gels, bars or granular forms.

Non-dusting granulates may be produced, e.g., as disclosed in US 4,106,991 and 4,661,452 (both to Novo Industri A/S) and may optionally be coated by methods known in the art Examples of waxy coating materials are poly(ethylene oxide) products (polyethyleneglycol, PEG) with mean molecular weights of 1000 to 20000; ethoxylated nonylphenols having from 16 to 50 ethylene oxide units; ethoxylated fatty alcohols in which the alcohol contains from 12 to 20 carbon atoms and in which there are 15 to 80 ethylene oxide units; fatty alcohols; fatty acids; and mono- and di- and triglycerides of fatty acids. Examples of film-forming coating materials suitable for application by fluid bed techniques are given in GB 1483591.

Granular compositions according to the present invention can also be in "compact form", i.e. they may have a relatively higher density than conventional granular detergents, i.e. form 550 to 950 g/l; in such case, the granular detergent compositions according to the present invention will contain a lower amount of "inorganic filler salt", compared to

conventional granular detergents; typical filler salts are alkaline earth metal salts of sulphates and chlorides, typically sodium sulphate; "Compact" detergent typically comprise not more than 10% filler salt. The liquid compositions according to the present invention can also be in "concentrated form", in such case, the liquid detergent compositions according to the present invention will contain a lower amount of water, compared to conventional liquid detergents. Typically, the water content of the concentrated liquid detergent is less than 30%, more preferably less than 20%, most preferably less than 10% by weight of the detergent compositions.

The compositions of the invention may for example, be formulated as hand and machine laundry detergent compositions including laundry additive compositions and compositions suitable for use in the pretreatment of stained fabrics, rinse added fabric softener compositions, and compositions for use in general household hard surface cleaning operations and dishwashing operations.

The following examples are meant to exemplify compositions for the present invention, but are not necessarily meant to limit or otherwise define the scope of the invention.

In the detergent compositions, the abbreviated component identifications have the following meanings:

LAS: Sodium linear $C_{12}$ alkyl benzene sulphonate

TAS: Sodium tallow alkyl sulphate

XYAS: Sodium $C_{1X}$ - $C_{1Y}$ alkyl sulfate

SS: Secondary soap surfactant of formula 2-butyl octanoic acid

25EY: A $C_{12}$ - $C_{15}$ predominantly linear primary alcohol condensed with an average of Y moles of ethylene oxide

45EY: A $C_{14}$ - $C_{15}$ predominantly linear primary alcohol condensed with an average of Y moles of ethylene oxide

XYEZS: $C_{1X}$ - $C_{1Y}$ sodium alkyl sulfate condensed with an average of Z moles of ethylene oxide per mole

Nonionic: $C_{13}$ - $C_{15}$ mixed ethoxylated/propoxylated fatty alcohol with an average degree of ethoxylation of 3.8 and an average degree of propoxylation of 4.5 sold under the tradename Plurafax LF404 by BASF Gmbh

CFAA: $C_{12}$ - $C_{14}$ alkyl N-methyl glucamide

TFAA: $C_{16}$ - $C_{18}$ alkyl N-methyl glucamide

Silicate: Amorphous Sodium Silicate ($SiO_2$:$Na_2O$ ratio = 2.0)

NaSKS-6: Crystalline layered silicate of formula $\delta$-$Na_2Si_2O_5$

Carbonate: Anhydrous sodium carbonate

Phosphate: Sodium tripolyphosphate

MA/AA: Copolymer of 1:4 maleic/acrylic acid, average molecular weight about 80,000

Polyacrylate: Polyacrylate homopolymer with an average molecular weight of 8,000 sold under the tradename PA30 by BASF Gmbh

Zeolite A: Hydrated Sodium Aluminosilicate of formula $Na_{12}(AlO_2SiO_2)_{12}.27H_2O$ having a primary particle size in the range from 1 to 10 micrometers

Citrate: Tri-sodium citrate dihydrate

Citric: Citric Acid

Perborate: Anhydrous sodium perborate monohydrate bleach, empirical formula $NaBO_2.H_2O_2$

PB4: Anhydrous sodium perborate tetrahydrate

Percarbonate: Anhydrous sodium percarbonate bleach of empirical formula $2Na_2CO_3.3H_2O_2$

TAED: Tetraacetyl ethylene diamine

CMC: Sodium carboxymethyl cellulose

DETPMP: Diethylene triamine penta (methylene phosphonic acid), marketed by Monsanto under the Tradename Dequest 2060

PVP. Polyvinylpyrrolidone polymer

EDDS: Ethylenediamine-N, N'-disuccinic acid, [S,S] isomer in the form of the sodium salt

Suds Suppressor: 25% paraffin wax Mpt 50°C, 17% hydrophobic silica, 58% paraffin oil

Granular Suds suppressor: 12% Silicone/silica, 18% stearyl alcohol, 70% starch in granular form

Sulphate: Anhydrous sodium sulphate

HMWPEO: High molecular weight polyethylene oxide

TAE 25: Tallow alcohol ethoxylate (25)

Detergent Example I

A granular fabric cleaning composition in accordance with the invention may be prepared as follows:

| | |
|---|---|
| Sodium linear $C_{12}$ alkyl benzene sulfonate | 6.5 |
| Sodium sulfate | 15.0 |
| Zeolite A | 26.0 |
| Sodium nitrilotriacetate | 5.0 |
| Enzymes (incl. endoglucanase) | 0.1 |
| PVP | 0.5 |
| TAED | 3.0 |
| Boric acid | 4.0 |
| Perborate | 18.0 |
| Phenol sulphonate | 0.1 |
| Minors | Up to 100 |

Detergent Example II

A compact granular fabric cleaning composition (density 800 g/l) in accord with the invention may be prepared as follows:

| | |
|---|---|
| 45AS | 8.0 |
| 25E3S | 2.0 |
| 25E5 | 3.0 |
| 25E3 | 3.0 |
| TFAA | 2.5 |
| Zeolite A | 17.0 |
| NaSKS-6 | 12.0 |
| Citric acid | 3 0 |
| Carbonate | 7 0 |
| MA/AA | 5.0 |
| CMC | 0.4 |
| Enzyme (Incl. endoglucanase) | 0.1 |
| TAED | 6.0 |
| Percarbonate | 22.0 |
| EDDS | 0.3 |
| Granular suds suppressor | 3.5 |
| water/minors | Up to 100% |

Detergent Example III

Granular fabric cleaning compositions in accordance with the invention which are especially useful in the laundering of coloured fabrics were prepared as follows:

| | | |
|---|---|---|
| LAS | 10.7 | - |
| TAS | 2.4 | - |
| TFAA | - | 4.0 |
| 45AS | 3.1 | 10.0 |
| 45E7 | 4.0 | - |
| 25E3S | - | 3.0 |
| 68E11 | 1.8 | - |
| 25E5 | - | 8.0 |
| Citrate | 15.0 | 7.0 |
| Carbonate | - | 10 |
| Citric acid | 2.5 | 3.0 |
| Zeolite A | 32.1 | 25.0 |
| Na-SKS-6 | - | 9.0 |
| MA/AA | 5.0 | 5.0 |
| DETPMP | 0.2 | 0.8 |
| Enzyme (incl. endo-glucanase) | 0.10 | 0.05 |
| Silicate | 2.5 | - |
| Sulphate | 5.2 | 3.0 |
| PVP | 0.5 | - |
| Poly (4-vinylpyridine)-N-Oxide/copolymer of vinyl-imidazole and vinyl-pyrrolidone | - | 0.2 |
| Perborate | 1.0 | - |
| Phenol sulfonate | 0.2 | - |
| Water/Minors | Up to 100% | |

Detergent Example IV

Granular fabric cleaning compositions in accordance with the invention which provide "Softening through the wash" capability may be prepared as follows:

| | | |
|---|---|---|
| 45AS | - | 10.0 |
| LAS | 7.6 | - |
| 68AS | 1.3 | - |
| 45E7 | 4.0 | - |
| 25E3 | - | 5.0 |
| Coco-alkyl-dimethyl hydroxy- | 1.4 | 1.0 |

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ethyl ammonium chloride

| | | |
|---|---|---|
| Citrate | 5.0 | 3.0 |
| Na-SKS-6 | - | 11.0 |
| Zeolite A | 15.0 | 15.0 |
| MA/AA | 4.0 | 4.0 |
| DETPMP | 0.4 | 0.4 |
| Perborate | 15.0 | - |
| Percarbonate | - | 15.0 |
| TAED | 5.0 | 5.0 |
| Smectite clay | 10.0 | 10.0 |
| HMWPEO | - | 0.1 |
| Enzyme (incl. endo-glucanase) | 0.10 | 0.05 |
| Silicate | 3.0 | 5.0 |
| Carbonate | 10.0 | 10.0 |
| Granular suds suppressor | 1.0 | 4.0 |
| CMC | 0.2 | 0.1 |
| Water/Minors | Up to 100% | |

Detergent Example V

Heavy duty liquid fabric cleaning compositions in accordance with the invention may be prepared as follows:

| | I | II |
|---|---|---|
| LAS acid form | - | 25.0 |
| Citric acid | 5 0 | 2.0 |
| 25AS acid form | 8.0 | - |
| 25AE2S acid form | 3.0 | - |
| 25AE7 | 8.0 | - |
| CFAA | 5 | - |
| DETPMP | 1.0 | 1.0 |
| Fatty acid | 8 | - |
| Oleic acid | - | 1.0 |
| Ethanol | 4.0 | 6.0 |
| Propanediol | 2.0 | 6.0 |
| Enzyme (incl.endo-glucanase) | 0.10 | 0.05 |
| Coco-alkyl dimethyl hydroxy ethyl ammonium | - | 3.0 |

| | | |
|---|---|---|
| chloride | | |
| Smectite clay | - | 5.0 |
| PVP | 2.0 | - |
| Water / Minors | Up to 100% | |

**Uses**

**Laundry**

The enzyme composition of the invention may be useful in a detergent composition for household or industrial laundering of textiles and garments, and in a process for machine wash treatment of fabrics comprising treating the fabrics during one or more washing cycle of a machine washing process with a washing solution containing the enzyme or enzyme preparation of the invention.

Typically, the detergent composition used in the washing process comprises conventional ingredients such as surfactants (anionic, nonionic, zwitterionic, amphoteric), builders, bleaches (perborates, percarbonates or hydrogen peroxide) and other ingredients, e.g. as described in WO 97/01629 which is hereby incorporated by reference in its entirety.

The endo-beta-1,4-glucanase of the invention provides advantages such as improved stain removal and decreased soil redeposition. Certain stains, for example certain food stains, contain beta-glucans which make complete removal of the stain difficult to achieve. Also, the cellulosic fibres of the fabrics may possess, particularly in the "non-crystalline" and surface regions, beta-glucan polymers that are degraded by this enzyme. Hydrolysis of such beta-glucans, either in the stain or on the fabric, during the washing process decreases the binding of soils onto the fabrics.

Household laundry processes are carried out under a range of conditions. Commonly, the washing time is from 5 to 60 minutes and the washing temperature is in the range 15 – 60°C, most commonly from 20 – 40°C. The washing solution is normally neutral or alkaline, most commonly with pH 7 – 10.5. Bleaches are commonly used, particularly for laundry of white fabrics. These bleaches are commonly the peroxide bleaches, such as sodium perborate, sodium percarbonate or hydrogen peroxide.

**MATERIALS & METHODS**

**Strains and donor organism**

The *Bacillus sp.* DSM 12648 mentioned above comprises the endo-beta-1,4-glucanase encoding DNA sequence shown in SEQ ID NO:1.

*B.subtilis* PL2306: This strain is the *B.subtilis* DN1885 with disrupted apr and npr genes (Diderichsen, B , Wedsted, U., Hedegaard, L., Jensen, B. R., Sjøholm, C. (1990) Cloning of aldB, which encodes alpha-acetolactate decarboxylase, an exoenzyme from *Bacillus brevis*. J. Bacteriol., 172, 4315-4321) disrupted in the transcriptional unit of the known *Bacillus subtilis* cellulase gene, resulting in cellulase negative cells. The disruption was performed essentially as described in Eds. A.L. Sonenshein, J.A. Hoch and Richard Losick (1993) *Bacillus subtilis* and other Gram-Positive Bacteria, American Society for microbiology, p.618.

Competent cells were prepared and transformed as described by Yasbin, R.E., Wilson, G.A. and Young, F.E. (1975) Transformation and transfection in lysogenic strains of *Bacillus subtilis*: evidence for selective induction of prophage in competent cells. J. Bacteriol, 121:296-304.

**General molecular biology methods**

Unless otherwise stated all the DNA manipulations and transformations were performed using standard methods of molecular biology (Sambrook et al. (1989) Molecular cloning: A laboratory manual, Cold Spring Harbor lab., Cold Spring Harbor, NY; Ausubel, F. M. et al. (eds.) "Current protocols in Molecular Biology". John Wiley and Sons, 1995; Harwood, C. R., and Cutting, S. M. (eds.) "Molecular Biological Methods for Bacillus". John Wiley and Sons, 1990).

Enzymes for DNA manipulations were used according to the manufacturer's instructions (e.g. restriction endonucleases, ligases etc. are obtainable from New England Biolabs, Inc.).

**Plasmids**

**pMOL944.** This plasmid is a pUB110 derivative essentially containing elements making the plasmid propagate in *Bacillus subtilis*, kanamycin resistance gene and having a strong promoter and signal peptide cloned from the amyL gene of *B.licheniformis* ATCC14580. The signal peptide contains a SacII site making it convenient to clone the DNA encoding the mature part of a protein in-fusion with the signal peptide. This results in the expression of a Preprotein which is directed towards the exterior of the cell.

The plasmid was constructed by means of ordinary genetic engineering and is briefly described in the following.

Construction of pMOL944:

The pUB110 plasmid (McKenzie, T. et al., 1986, Plasmid 15:93-103) was digested with the unique restriction enzyme NciI. A PCR fragment amplified from the amyL promoter

encoded on the plasmid pDN1981 (P.L. Jørgensen et al., 1990, Gene, 96, p37-41.) was digested with NciI and inserted in the NciI digested pUB110 to give the plasmid pSJ2624.

The two PCR primers used have the following sequences:

# LWN5494 (SEQ ID NO:5)

5'-GTCGCCGGGGCGGCCGCTATCAATTGGTAACTGTATCTCAGC -3'

# LWN5495 (SEQ ID NO:6)

5'-GTCGCCCGGGAGCTCTGATCAGGTACCAAGCTTGTCGACCTGCAGAA TGAGGCAGCAAGAAGAT -3'

The primer #LWN5494 inserts a NotI site in the plasmid.

The plasmid pSJ2624 was then digested with SacI and NotI and a new PCR fragment amplified on amyL promoter encoded on the pDN1981 was digested with SacI and NotI and this DNA fragment was inserted in the SacI-NotI digested pSJ2624 to give the plasmid pSJ2670.

This cloning replaces the first amyL promoter cloning with the same promoter but in the opposite direction. The two primers used for PCR amplification have the following sequences:

#LWN5938 (SEQ ID NO:7)

5'-GTCGGCGGCCGCTGATCACGTACCAAGCTTGTCGACCTGCAGAATG AGGCAGCAAGAAGAT -3'

#LWN5939 (SEQ ID NO:8)

5'-GTCGGAGCTCTATCAATTGGTAACTGTATCTCAGC -3'

The plasmid pSJ2670 was digested with the restriction enzymes PstI and BclI and a PCR fragment amplified from a cloned DNA sequence encoding the alkaline amylase SP722 (Patent # WO9526397-A1) was digested with PstI and BclI and inserted to give the plasmid pMOL944. The two primers used for PCR amplification have the following sequence:

#LWN7864 (SEQ ID NO:9)

5' -AACAGCTGATCACGACTGATCTTTTAGCTTGGCAC-3'

#LWN7901 (SEQ ID NO:10)

5' -AACTGCAGCCGCGGCACATCATAATGGGACAAATGGG -3'

The primer #LWN7901 inserts a SacII site in the plasmid

**Genomic DNA Preparation**

The strain DSM 12648 was propagated in liquid medium 2xTY containing 1% carboxymethyl-cellulose + (0,1M Na2CO3 + 0,1M NaHCO3 separately autoclaved and added aseptically after cooling to room temperature). After 16 hours of incubation at 30°C and 300

rpm, the cells were harvested, and genomic DNA was isolated by the method described by *Pitcher et al.* [*Pitcher, D. G., Saunders, N. A., Owen, R. J;* Rapid extraction of bacterial genomic DNA with guanidium thiocyanate; Lett Appl Microbiol 1989, 8:151-156].

**Media**

**TY** (as described in Ausubel, F. M. et al. (eds.): "Current protocols in Molecular Biology", John Wiley and Sons, 1995).

**2xTY** (as described in Ausubel, F. M. et al. (eds.): "Current protocols in Molecular Biology", John Wiley and Sons, 1995).

**LB agar** (as described in Ausubel, F. M. et al. (eds.): "Current protocols in Molecular Biology", John Wiley and Sons, 1995).

**LBPG** is LB agar supplemented with 0.5% Glucose and 0.05 M potassium phosphate, pH 7.0

**AZCL-HE-cellulose** is added to LBPG-agar to 0.5 % AZCL- HE-cellulose is from Megazyme, Australia.

**BPX media** is described in EP 0 506 780 (WO 91/09129).

**Cal 18-2 media** is described in patent application WO 00/75344 A1).

**Test for LAS stability**

The endo-glucanase/cellulase is incubated with different concentrations of LAS (linear alkyl benzene sulphonate; Nansa 1169/P) for 10 min at 40°C. The residual activity is determined using the ECU method described in the Materials & Methods section.

LAS is diluted in 0.1 M phosphate buffer pH 7.5.
The following concentrations is used.
500 ppm, 250 ppm, 100 ppm, 50ppm, 25ppm, and 10 ppm on no LAS.

The endo-glucanase is diluted in the different LAS buffers to 0.2 S-CEVU/ml final concentration in a total volume of 10 ml and incubated for 10 min in a temperature controlled water bath.

Then the residual activity is determined in duplicate using the ECU method.

The two samples of 0.5 ml solution were mixed with 1.5 ml 1% CMC solution (Hercules 7L) prepared in the same phosphate buffer, incubation for 20 min at 40°C, and then stopped with PHBAH, sodium tartrate in 2% NaOH.

The similar blank sample of 0.5 ml was added to the CMC solution after addition of stop reagent.

The samples are cooked for 10 min and the absorbance is measured at 410 nm.

The activity is measured after subtraction of the blank.

The activity with no LAS was 100%.

**Determination of endo-beta-1,4-glucanase activity**

**ECU method**

In the ECU method the ability of the enzyme sample to reduce the viscosity of a solution of carboxymethyl-cellulose (CMC) is determined, and the result is given in ECU. The reduction in viscosity is proportional to the endo-cellulase activity. Conditions: CMC type 7LFD from Hercules, pH 7.5 in 0.1M phosphate buffer, CMC concentration 31.1 g per liter reaction at 40°C for 30 minutes. A vibration viscosimeter such as MIVI 3000, Sofraser, France is used to measure the viscosity.

**Cellazyme C method**

Cellazyme C is an endo-glucanase assay substrate, supplied in tablet form by Megazyme International Ireland Ltd. Reference is made to Megazyme's pamphlet CZC 7/99 which states: "The substrate is prepared by dyeing and cross-linking HE-cellulose to produce a material which hydrates in water but is water insoluble. Hydrolysis by endo-beta-1,4-glucanase produces water-soluble dyed fragments, and the rate of release of these (increase in absorbance at 590nm) can be related directly to enzyme activity."

The enzyme sample is added to 6ml of a suitable buffer in a test tube, one Cellazyme C tablet is added and dispersed by shaking the tube, then the tube is placed in a water bath at 40°C. The contents are mixed by brief shaking after approximately 15, 30, 45 and 60 minutes After 60 minutes the solution is filtered through Whatman GF/C filters, 9cm diameter. The absorbance of the filtered solution is measured at 590nm.

**Determination of Mannanase activity**

Mannanase activity may be tested for by applying a solution to be tested to 4 mm diameter holes punched out in agar plates containing 0.2% AZCL galactomannan (carob), i.e. substrate for the assay of endo-1,4-beta-D-mannanase available as CatNo.I-AZGMA from the company Megazyme (Megazyme's Internet address: http://www.megazyme.com/Purchase/index.html).

The following examples illustrate the invention.

**EXAMPLE 1**

**Cloning and expression of endo-beta-1,4-glucanase gene from *Bacillus sp.***

Sub-cloning and expression of mature endo-glucanase in *B.subtilis.*

The endo-glucanase encoding DNA sequence of the invention was PCR amplified using the PCR primer set consisting of these two oligo-nucleotides:

# 168684 (SEQ ID NO:11)
5'-CAT TCT GCA GCC GCG GCA GCA GAA GGA AAC ACT CGT GAA GAC-3'

# 168685 (SEQ ID NO:12)
5'-GCG TTG AGA CGC GCG GCC GCT TAC TCT TCT TTC TCT TCT TTC TC-3'

Restriction sites SacII and NotI are underlined.

The oligonucleotides were used in a PCR reaction in HiFidelityTM PCR buffer (Boehringer Mannheim, Germany) supplemented with 200 micro M of each dNTP, 2.6 units of HiFidelityTM Expand enzyme mix and 200 pmol of each primer. Chromosomal DNA isolated from *Bacillus sp.* DSM12648 as described above was used as template.

The PCR reaction was performed using a DNA thermal cycler (Landgraf, Germany). One incubation at 94°C for 1 min followed by ten cycles of PCR performed using a cycle profile of denaturation at 94°C for 15 sec, annealing at 60°C for 60 sec, and extension at 72°C for 120sec, followed by twenty cycles of denaturation at 94°C for 15 sec, 60°C for 60 sec and 72°C for 120 sec (at this elongation step 20 sec are added every cycle). 5 µl aliquots of the amplification product was analysed by electrophoresis in 0.7 % agarose gels (NuSieve, FMC). The appearance of a DNA fragment size 2.4 kb indicated proper amplification of the gene segment.

Subcloning of PCR fragment:

45 microL aliquots of the PCR products generated as described above were purified using QIAquick PCR purification kit (Qiagen, USA) according to the manufacturer's instructions. The purified DNA was eluted in 50 microL of 10mM Tris-HCl, pH 8.5.
5 µg of pMOL944 and 25 microL of the purified PCR fragment was digested with SacII and NotI, electrophoresed in 0.7 % agarose gels (NuSieve, FMC), the relevant fragments were excised from the gels, and purified using QIAquick Gel extraction Kit (Qiagen, USA) according to the manufacturer's instructions. The isolated PCR DNA fragment was then ligated to the SacII-NotI digested and purified pMOL944. The ligation was performed overnight at 16°C us-

ing 0.5 micro g of each DNA fragment, 1 U of T4 DNA ligase and T4 ligase buffer (Boehringer Mannheim, Germany).

The ligation mixture was used to transform competent *B. subtilis* PL2306. The transformed cells were plated onto LBPG-10 micro g/ml of kanamycin-agar plates. After 18 hours incubation at 37°C colonies were seen on the plates. Several clones were analyzed by isolating plasmid DNA from overnight culture broths.

One such positive clone was re-streaked several times on agar plates as used above; this clone was called MB1181-7. The clone MB1181-7 was grown overnight in TY-10microg/mL kanamycin at 37°C, and next day 1 ml of cells were used to isolate a plasmid from the cells using the Qiaprep Spin Plasmid Miniprep Kit #27106 according to the manufacturers recommendations for *B. subtilis* plasmid preparations. This DNA was sequenced and revealed a DNA sequence identical to the endo-glucanase gene in SEQ ID NO:1 bp 1- 2322 encoding the mature endo-glucanase. The derived protein sequence is represented in SEQ ID NO: 2.

**EXAMPLE 2**

**Expression and recovery of the endo-glucanase from *Bacillus sp.* DSM 12648**

MB1181-7 obtained as described in Example 1 was grown in 15 x 200 ml Cal-18-2 media with 10 microg/mL of kanamycin, in 500 mL two-baffled shake flasks, for 4 days at 37°C at 300 rpm, whereby about 2500 ml of culture broth was obtained. The culture fluid was flocculated by adding 50% $CaCl_2$ (10 ml per liter of culture broth) together with 11% sodium aluminate (10 mL per liter of culture broth), maintaining the pH between 7.0 and 7.5 by adding 20% formic acid. Cationic agent Superfloc C521 (25 mL of a 10% v/v dilution per liter of culture broth) and anionic agent Superfloc A130 (75 ml of a 0.1% w/v dilution in water per liter of culture broth) was added during agitation to complete the flocculation. The flocculated material was separated by centrifugation using a Sorval RC 3B centrifuge at 10000 rpm for 30 min at 6°C. The resulting supernatant contained the endo-glucanase activity.

The supernatant was clarified using Whatman glass filters GF/D and C. Then ultra-filtration was used to concentrate and reduce the ionic strength of the solution. The ultra-filtration membrane was Filtron UF with a cut-off of 10 kDa. After ultra-filtration the solution had conductivity < 3mS/cm. The pH was adjusted to pH 8.0.

Anion-exchange chromatography on Q-Sepharose was then used for additional purification. The solution from ultra-filtration was applied to a 300 mL column containing Q-Sepharose (Pharmacia) equilibrated with a buffer of 25 mmol Tris pH 8.0. The endo-glucanase bound to the Q-Sepharose, and was then eluted using a 0.5 M NaCl gradient. The fractions with high endo-glucanase activity were pooled. The endo-glucanase activity of the final pooled endo-glucanase solution was approximately 1000 ECU per mL.

**EXAMPLE 3**

**Stain removal and anti-redeposition effect**

This test demonstrates the stain removal and anti-redeposition effects of the glucanase obtained in Example 2. Additionally this test demonstrates that the enzyme performance is essentially unchanged when sodium perborate bleach is included.

Cotton swatches are stained with beta-glucan (from barley) plus carbon black. Soiled swatches are washed together with clean swatches. After washing the swatches are rinsed and dried. The soil removal from the soiled switches and the soil redeposition onto the clean swatches is determined by reflectance measurements. The soil removal and soil redeposition after washing without or with addition of the endo-glucanase are compared.

Swatches: Cut from 100% cotton fabric, type #2003 (Tanigashira, Osaka, Japan), pre-washed at 40°C as a precaution to remove any water soluble contaminations, size 5x5cm, weight approximately 0.3g.

Washing equipment: Stirred beakers, beaker volume 250 ml, with temperature control by water bath heating. The equipment is a multi-beaker miniature agitator washer.

Detergent solution: Prepared by adding the following into deionised water.

Sodium carbonate, 0.5 g per liter

Sodium bicarbonate, 0.7 g per liter

$Ca^{2+}/Mg^{2+}$, to give water hardness 12°dH

Anionic surfactant, Surfac SDBS80 (sodium alkylbenzene sulphonate), 0.5 g per liter

Nonionic surfactant, Berol 537 (Akzo Nobel), 1.0 g per liter

Sodium perborate, type SPB from wfk Testgewebe, either 0 or 1.0 g per liter

Solution pH is approximately 9.5.

Washing procedure: 100mL detergent solution is added to each beaker. The water bath temperature is 40°C. The mechanical agitators are operated at approximately 125 rpm. The detergent solutions are pre-warmed for 10 minutes and then the endo-glucanase and the swatches are added. In each case three soiled swatches (prepared as described below) and three clean swatches are added to each beaker After washing for 30 minutes, the swatches are removed from the detergent solution, rinsed under running tap water for 5 minutes, spread flat on absorbent paper and allowed to dry.

Reflectance measurements: Made using a Macbeth 7000 Color Eye reflectance spectrophotometer. In the case of the soiled swatches, each swatch is measured once in the center of the soiled area, then the average value is calculated. In the case of the clean swatches, each swatch is measured once on each side, then the average value is calculated. The reflectance measurements are all made at 500nm.

Soiled swatches: Soiled swatches are made using beta-glucan (from barley) and carbon black ("carbon for detergency tests" supplied by Sentaku Kagaku Kyokai, Tokyo, Japan). Dissolve about 0.67g of beta-glucan in 100 mL tap water by stirring and warming to >50°C. Add 0.33g carbon black. Blend with an UltraTurrax T25 blender, speed 4000 rpm for 2 minutes. Apply 250 microLl of the beta-glucan/carbon onto the center of each swatch. Allow to dry overnight at room temperature.

The swatches used in this example had an average reflectance value of 93.5 before soil application and 17.5 after soiling.

Endo-glucanase addition: The endo-glucanase from Example 2 was added to give an activity concentration of 0, 20 or 100 ECU per liter of detergent solution.

Results: Detergent without bleach (average of reflectance measurements after washing)

| Endo-glucanase added | Soiled swatches | Clean swatches |
|---|---|---|
| 0 | 25.1 | 33.5 |
| 20 ECU per liter | 35.7 | 46.7 |
| 100 ECU per liter | 40.2 | 59.1 |

Results: Detergent with bleach (average of reflectance measurements after washing)

| Endo-glucanase added | Soiled swatches | Clean swatches |
|---|---|---|
| 0 | 24.6 | 27.7 |
| 20 ECU per liter | 36.8 | 52.6 |
| 100 ECU per liter | 39.3 | 63.2 |

The endo-glucanase increased the removal of soil from the fabric, as seen by the increased reflectance value of the stained swatches after washing with endo-glucanase as compared to the result after washing without endo-glucanase. The endo-glucanase also decreases the soil redeposition, as seen by the increased reflectance value of the clean swatches after washing with endo-glucanase. The improvements of soil removal and anti-redeposition provided by the endo-glucanase are essentially unchanged by the addition of the bleach.

**EXAMPLE 4**

**Anti-redeposition effect**

Clean cotton fabric is washed together with soiled cotton fabric in a solution of a household detergent. The wash is carried out in a Terg-O-Tometer. During the wash, soil is released from the soiled fabric into the detergent liquor. This soil can then redeposit onto the

clean cotton. After washing, the cotton fabrics are rinsed and dried, and then measured with a reflectance spectrophotometer in order to detect the degree of soil redeposition.

Detergent: Powder household detergent, Asian.

Detergent concentration: 0.67g/L in water with hardness 4°dH.

1000 mL of detergent solution per T-O-T beaker.

Cotton fabric: Total of 33g fabric per T-O-T beaker, comprising suitably sized pieces of:

white woven cotton, #2003 (Tanigashira, Osaka, Japan), total weight 11g

white cotton interlock, total weight 13g

soiled cotton fabric, type EMPA101 (EMPA, Switzerland), total weight 9g.

Wash: Temperature 25°C, wash time 40 minutes, at 125 rpm. After washing the #2003 cotton is rinsed under running tap water for 10 minutes, then dried.

Reflectance measurements. The pieces of #2003 woven cotton are measured, on both sides, using a Macbeth 7000 reflectance spectrophotometer, 500nm. The average result for measurements from each T-O-M beaker is calculated.

Enzyme addition: In this trial, the glucanase prepared as described in Example 2 was added to the detergent liquor before the start of the wash step.

Results:

| Glucanase added, ECU per liter | Reflectance of #2003, at 500nm |
|---|---|
| 0 | 76.67 |
| 0 | 76.05 |
| 1 | 81.86 |
| 5 | 84 30 |
| 20 | 84.85 |
| 50 | 85.99 |

From the results it can be concluded that addition of the endo-glucanase reduces the soil redeposition

**Example 5**

**LAS stability**

The LAS stability of the endo-glucanase prepared in Example 2 is tested as described in the Materials & Methods section.

**Example 6**

The tests in Example 4 and 5 is repeated with a combination of the endoglucanase prepared in Example in combination with the *Thielavia terrestris* variant disclosed in Example 1 of WO 98/12307.

5

**CLAIMS**

1. A detergent composition comprising an endo-glucanase, wherein the endo-glucanase is selected from one of

(i) the endo-glucanase having the amino acid sequence of position 1 to position 773 of SEQ ID NO: 2;

(ii) an endo-glucanase having a sequence of at least 70% identity to the amino acid sequence of position 1 to position 773 of SEQ ID NO:2; or a fragment thereof that has glucanase activity, when identity is determined by GAP provided in the GCG program package using a GAP creation penalty of 3.0 and GAP extension penalty of 0.1.

2. A detergent composition comprising anionic tensides and a combination of an endo-glucanase as described in Claim 1 and a fungal cellulase, wherein both enzymes are stable in the presence of anionic tensides.

3. A detergent composition of claim 1 or 2, wherein

(a) the endo-glucanase is selected from one of

(i) the endo-glucanase having the amino acid sequence of position 1 to position 773 of SEQ ID NO: 2;

(ii) an endo-glucanase having a sequence of at least 70% identity to the amino acid sequence of position 1 to position 773 of SEQ ID NO:2; or a fragment thereof that has glucanase activity, when identity is determined by GAP provided in the GCG program package using a GAP creation penalty of 3.0 and GAP extension penalty of 0.1.

(b) the cellulase is selected from one of

(i) the cellulase having the amino acid sequence of position 1 to position 299 of SEQ ID NO: 4 or

(ii) a cellulase having a sequence of at least 70% identity to the amino acid sequence of position 1 to position 299 of SEQ ID NO:4, or a fragment thereof that has cellulase activity, when identity is determined by GAP provided in the GCG program package using a GAP creation penalty of 3.0 and GAP extension penalty of 0.1.

4. The detergent composition of claims 1 to 3, wherein the endo-glucanase is derived from Bacillus sp. KSM-S237 (FERM P-165067) or comprises a polypeptide endogeneous to *Bacillus sp,* DSM 12648 shown in SEQ ID NO: 2.

5. The detergent composition of claims 1 to 4, wherein the endo-glucanase is active at a pH at least in the range of 4-11, preferably 5.5-10.5.

6. The detergent composition of claims 2 to 5, wherein cellulase is derived from a strain of the genus *Thielavia*, preferably a strain of *Thielavia terrestris*, especially *Thielavia terrestris* NRRL 8126 and shown in SEQ ID NO: 4.

7. The composition of claims 1 to 6, wherein the composition further comprises one or more enzymes selected from the group consisting of proteases, cellulases, beta-glucanases, hemicellulases, lipases, peroxidases, laccases, alpha--amylases, glucoamylases, cutinases, pectinases, reductases, oxidases, phenoloxidases, ligninases, pullulanases, pectate lyases, xyloglucanases, xylanases, pectin acetyl esterases, polygalacturonases, rhamnogalacturonases, pectin lyases, other mannanases, pectin methylesterases, cellobiohydrolases, transglutaminases; or mixtures thereof.

8. The composition of claims 1 to 7, wherein the protease is derived from a strain of Bacillus, preferably where the protease is a subtilisin selected from the group of subtilisin Novo, subtilisin Carlsberg, subtilisin 309, subtilisin 147 and subtilisin 168.

9. The composition of claim.1 to 8, wherein the lipase is derived from a strain of the genus *Humicola*, preferably a strain of *Humicola lanuginose*, especially *Humicola lanuginose* DSM4109.

10. The composition of claims 1 to 9, wherein the alpha-amylase is derived from a strain of the genus *Bacillus*, preferably a strain of *Bacillus* sp., especially *Bacillus* sp. DSM 12649, NCIB 12512 or NCIB 12513.

11. The composition of claims 1 to 10, wherein the mannanase is derived from a strain of the genus *Bacillus*, preferably *Bacillus licheniformis*, especially *Bacillus licheniformis* sp. I633

12. The composition of claims 1 to 11, wherein the pectate lyase is derived from a strain of the genus *Bacillus*, preferably *Bacillus subtilis*, especially *Bacillus subtilis* DSM14218

13. The composition of claim 1 to 12, wherein the cellulase is derived from a strain of the genus *Humicola*, preferably *Humicola insolens*, especially *Humicola insolens* DSM 1800.

14. A process for washing a fabric, comprising contacting a fabric with an aqueous solution of a composition of claims 1 to 13 under agitation for an effective period of time.

15. The process of claim 14, wherein the period of time is between 2 minutes and 2 hours, preferably 10 minutes to 60 minutes.

16. A process of claim 15, wherein the weight ratio of the endo-glucanase protein component to the total enzyme protein is less than 1:2.

**ABSTRACT**

The invention relates to a detergent composition comprising an endo-glucanase having anti-redeposition effect.

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10383.ST25

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